How to Analyze Drugs Using X-ray Diffraction

Drugs – An Introduction

This tutorial will cover the following topics

- Tablets and Powders
- Basic components of a formulated drug
- Identification of each component in a formulation
- Tips for an accurate identification

This tutorial does not cover the following

- The steps for identifying phases using SIeve+

http://www.icdd.com/resources/tutorials/pdf/SIeveplusTutorial.pdf

- How to display and plot simulations of the identification solutions

http://www.icdd.com/resources/tutorials/pdf/Digital%20Pattern%20Simulation.pdf

Tablets and Powders

Drugs come in a variety of forms (tablets, pills, capsules, aerosol sprays) and with a variety of formulations. A single drug may be formulated in dozens of ways to enhance the ability of the drug to enter your body (fast relief or long-lasting relief) and be delivered to a targeted area (skin cream, nasal spray, anti-itch, anti-cancer). A formulation could help shelf-life, provide buffering capability in your stomach, make the medicine taste better, or be part of a design for time-release of the drug into your bloodstream.

The first question to ask is what problem are you trying to solve? Do you want to identify the ingredients or understand the effectiveness of a drug coating or packaging material? Are you trying to determine effective shelf life? X-ray diffraction analyses are used in all these applications.

Tablets and Powders


[1]


[2]


[3]

The classic method for drug analysis is to take the formulated pill, capsule or tablet [1]. Crush the contents in a mortar and pestle [2] or a grinding mill*. Then place the resulting fine powder into a cavity depression mount [3] suitable for an X-ray diffraction experiment. This is a good method for identifying the ingredients in a formulation and was the method used for most of the data shown in this tutorial. The specimens shown in [3] are all commercial pharmaceutical formulations in powder form.

If sample sizes are limited, many professionals in the field use capillary mounts and a diffractometer with transmission geometry that can focus on the small specimen size.

*Most drugs are soft materials and there are several documented cases of phase transformations that have occured with mechanical grinding. A light grind with a mortar and pestle is often preferred.

Tablets and Powders

Courtesy of Shimazdu

With today's X-ray diffraction instrumentation and accessories, tablets and capsules can be analyzed directly without grinding. Accessories like microfocus X-ray tubes, collimating optics and specially designed X-ray masks can allow the user to focus the X-ray beam on the surface of the pill. With sophisticated instruments that allow the angle of incidence of the X-ray beam to vary, especially at low angles, the user may be able to generate a depth profile of ingredients within the tablet without having to grind the tablet.

These types of analyses can be used to determine whether the ingredients are uniformly distributed within the tablet. It is usually important that pigments and lubricants are concentrated on the surface. Lubricants such as stearic acid and various stearates are used in today's high speed tablet presses. The distribution and type of phases may demonstrate whether the surface is being preferentially changed due to the effects of temperature and humidity, influencing shelf life. Instrumentation accessories may include temperature and humidity chambers to examine tablet shelf life under controlled conditions.

References

Morning	Amorphous, Activated and Nanomaterials **Session Chair: Dr. Julien Giovannini,** AstraZeneca, Nottingham, U.K.	
Type	**Title of Paper**	**Speaker & Affiliation**
Invited	**PHYSICAL STABILITY OF AMORPHOUS SOLID DISPERSIONS: XRPD AND COMPUTATIONAL STUDIES OF 'MISCIBILITY'** (.pdf)	**Igor Ivanisevic,** SSCI, a division of APTUIT Inc., West Lafayette, IN, U.S.A.
Invited	**CRYSTALLITE SIZES AND MICROSTRAINS DETERMINED BY RIETVELD REFINEMENT: APPLICATION TO MILLED LACTOSE** (.pdf)	**Vincent Caron,** Trinity College Dublin, Dublin, Ireland
Contributed	**CHARACTERISATION AND PREDICTION OF STABILITY OF AMORPHOUS MATERIALS DURING PHARMACEUTICAL DEVELOPMENT: PAIR-WISE DISTRIBUTION FUNCTION** (.pdf)	Helen Blade, AstraZeneca, Macclesfield, Cheshire, U.K.
Contributed	**DIFFRACTION, NON-CRYSTALLINITY, AND THE PDF DATABASE** (.pdf)	**Cyrus Crowder,** ICDD, Newtown Square, PA, U.S.A.
Contributed	**STUDYING AMORPHOUS PHARMACEUTICAL MATERIALS BY POWDER X-RAY DIFFRACTION AND OTHER SOLID-STATE TECHNIQUES** (.pdf)	**Shawn Yin,** Birstol-Myers Squibb, New Brunswick, NJ, U.S.A.
Contributed	**QUANTITATIVE ANALYSIS OF PHASES WITH PARTIAL OR NO KNOWN CRYSTAL STRUCTURE** (.pdf)	Geert Vanhoyland, Bruker AXS GmbH, Karlsruhe, Germany

If you want to find out more about the advanced application of X-ray diffraction in drug analysis, the ICDD holds an annual pharmaceutical symposium PPXRD at various locations around the world. Several presentations from past symposia are available for free download on the icdd.com website

http://www.icdd.com/resources/researchtools.htm

An example of the presentations are available on the website.

The rest of this tutorial will focus on the fundamentals involved in drug and formulation material identification.

Formulations

- The drug is commonly referred to as the “active substance” or **API** which is an abbreviation for “Active Pharmaceutical Ingredient”.
- The formulation often contains lubricants, fillers, flavors, disintegrates, binders and colored pigments. Pigments are frequently used to hide the brown or yellow colors of the ingredients and make the tablet pleasing to the eye. They are also frequently used to differentiate different dosage strengths of the same drug. Various sugars and waxes may be used to mask an unpleasant taste or make the pill chewable for children. Buffers might be added to control the pH so that the tablet dissolves in the stomach lining or intestines in a controlled manner. As a collective group these materials are known as “**excipients**”.
- Most formulations contain multiple excipients with each excipient serving a specific purpose.
- Excipients are commonly defined as inactive ingredients that serve multiple functions in drug delivery. If the tablet is for human consumption then the excipients are usually regulated. ICDD follows USP (United States Pharmacopeia) European and National Formulary definitions of excipients for materials in the excipient subfile.
- In general, excipients are the bulk contents of any formulation and the API may be in small concentrations. It is usually necessary to analyze and identify all the excipients before one can make an accurate identification of the API.

Formulations

Supplement Facts

Serving Size 1 Caplet

	Amount Per Caplet	% Daily Value
Vitamin A (as vitamin A acetate & beta carotene)	3,500 IU	70%
Vitamin C (as ascorbic acid)	60 mg	100%
Vitamin D (as cholecalciferol)	400 IU	100%
Vitamin E (as dl-alpha tocopheryl acetate)	30 IU	100%
Vitamin K (as phytonadione)	25 mcg	31%
Thiamine (vitamin B_1)(as thiamine mononitrate)	1.5 mg	100%
Riboflavin (vitamin B_2)	1.7 mg	100%
Niacin (as niacinamide)	20 mg	100%
Vitamin B_6 (as pyridoxine hydrochloride)	2 mg	100%
Folate (as folic acid)	400 mcg	100%
Vitamin B_{12} (as cyanocobalamin)	6 mcg	100%
Biotin	30 mcg	10%
Pantothenic acid (as d-calcium pantothenate)	10 mg	100%
Calcium (as dicalcium phosphate & calcium carbonate)	162 mg	16%
Iron (as ferrous fumarate)	18 mg	100%
Phosphorus (as dicalcium phosphate)	109 mg	11%
Iodine (as potassium iodide)	150 mcg	100%
Magnesium (as magnesium oxide)	100 mg	25%
Zinc (as zinc oxide)	15 mg	100%
Selenium (as sodium selenate)	20 mcg	29%
Copper (as cupric oxide)	2 mg	100%
Manganese (as manganese sulfate)	2 mg	100%
Chromium (as chromium chloride)	120 mcg	100%
Molybdenum (as sodium molybdate)	75 mcg	100%
Chloride (as potassium chloride)	72 mg	2%
Potassium (as potassium chloride)	80 mg	2%
Boron (as sodium borate)	150 mcg	*
Nickel (as nickel sulfate)	5 mcg	*
Silica (as silicon dioxide)	2 mg	*
Tin (as tin chloride)	10 mcg	*
Vanadium (as sodium metavanadate)	10 mcg	*
Lycopene (from tomato extract (fruit))	300 mcg	*
Lutein	250 mcg	*

*Daily Value not established.

In most countries, manufacturers are required to list the ingredients. Such a listing is provided on the left for a Centrum® vitamin pill. A single pill is typically 1400 mg and the list accounts for ~660 mg.

There is an additional sentence that accompanies the list that states, “Other ingredients dicalcium phosphate, microcrystalline cellulose, ginko biloba leaf extract, gelatin, ginseng root, crospovidone, starch, red 40 aluminum lake”.

Most of the above materials are the lubricants, pigments, buffering agents, fillers and flavors that are the excipients of the formulation and account for the remaining weight of the pill.

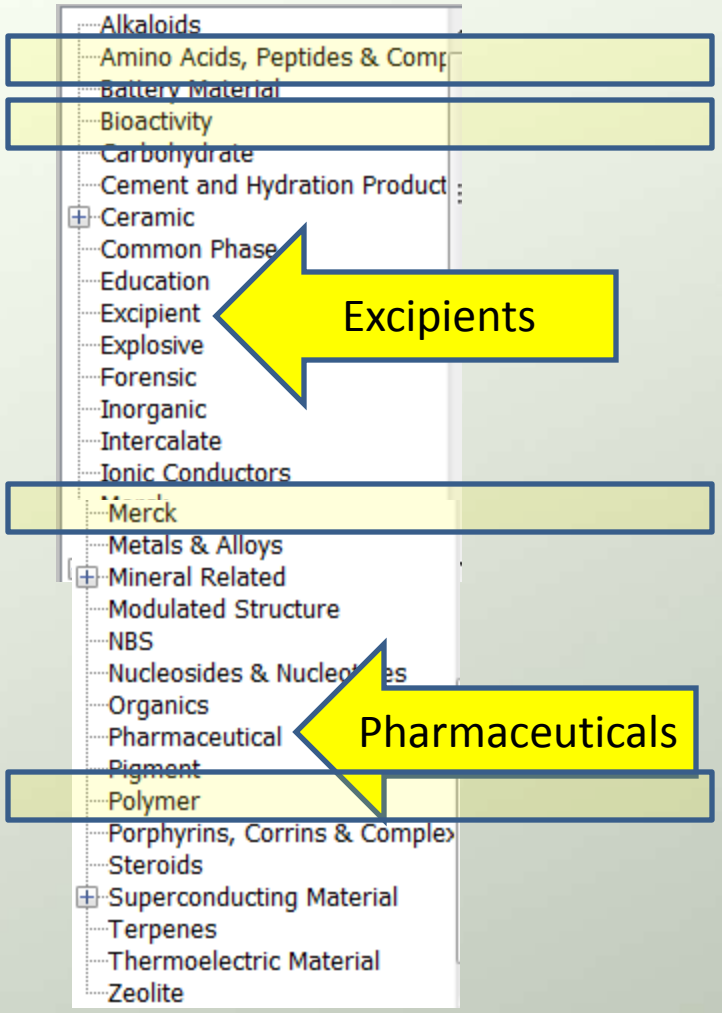
This is a very complex formulary and an X-ray diffraction analysis would only be expected to identify the major ingredients above 5 weight %, unless specific steps were taken to improve the sensitivity. Examples of the latter include long data collection times, use of high resolution optics or use of an intense X-ray source such as a synchrotron. Recent advances in detector technology are resulting in a higher dynamic ranges and improved sensitivity to low count rates.

Guidelines for a Drug Formulation Analysis

- The major phases should be excipients. Excipients are specified materials that may be organic, inorganic or polymeric.
- Most formulations contain multiple excipients, each of which have a specific function in drug delivery. (Identify the material and then use the identification to identify the function).
- If the active ingredient (API) is a potent drug, it might be present in small concentration. Find the excipient first, then analyze for the API.

Use Subfiles

Subfiles in ICDD databases



Ingredients of a tablet are specified substances. ICDD classifies them according to function. If the tablet is for human consumption then the excipient subfile and pharmaceutical subfile have the desired entries.

The other highlighted subfiles may be helpful in related studies. Amino acids and peptides are common in health supplements. The polymer subfile might be useful to analyze packaging. The Merck subfile is a collection of materials found in pharmaceutical production and manufacturing industry.

Subfile Content in ICDD Products

	Pharmacueticals	Excipients	Bioactives	Total
PDF-4/Organics Release 2012	8,007	2,080	14,245	471,257
PDF-4+ Release 2011	6,719	2,094	382	328,660
PDF-2 Release 2011	6,312	1,857	382	250,182

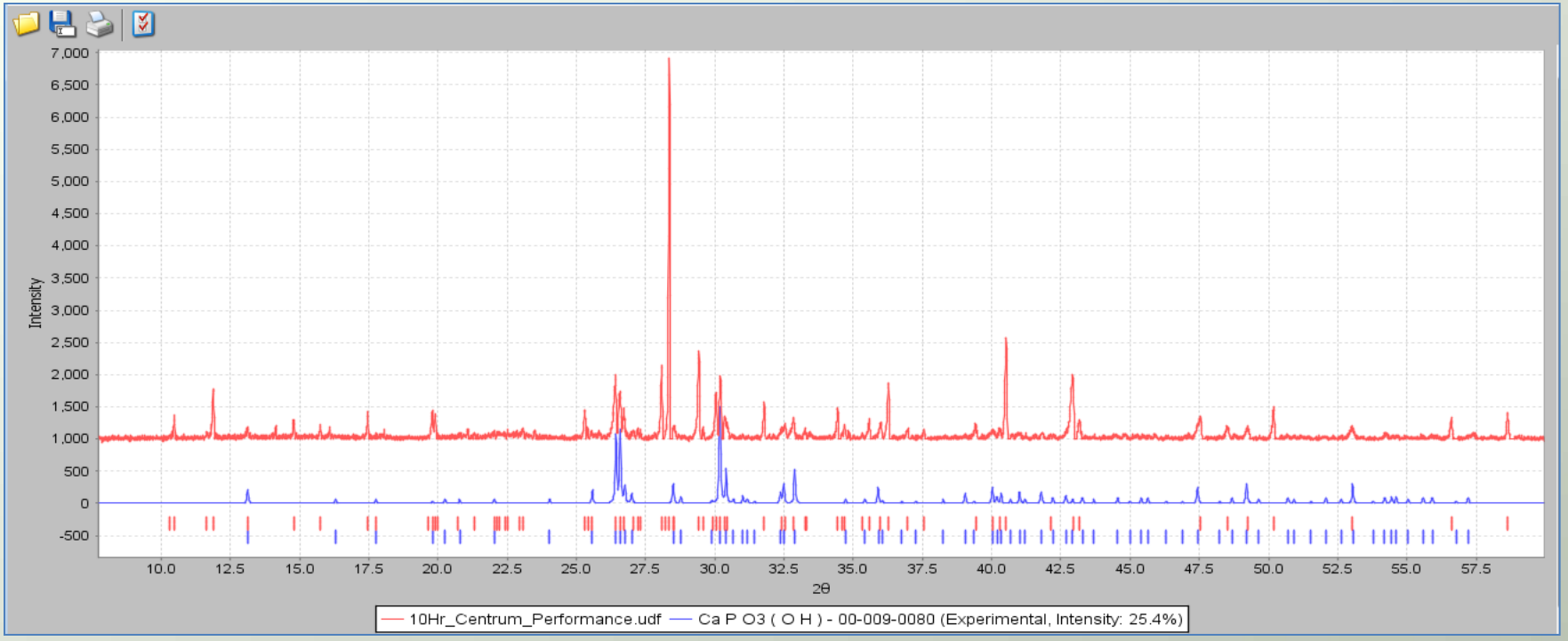
Bioactives are materials that show bioactivity at the time of publication. Many become pharmaceutical API's with the passage of time. Of these three major databases, PDF-4/Organics is the most powerful relative to comprehensive material identification of drugs and drug formulations. Bioactives also contains herbicides, pesticides and other commercial materials but not for human consumption.

Subfiles

ICDD software and all commercial Search/Match and material identification software have the capability for the user to select one or more subfiles during a search.
This is a very powerful filter that can increase the accuracy of your analysis. Essentially, you are limiting your search to only those chemistries in a tablet that are possible with the specimen that you are analyzing. Complex formulations have dozens, if not hundreds, of diffraction peaks and the PDF databases has tens of millions of reference d-spacings. The PDF-4/Organics database has over 88 million d-spacings in Release 2012.

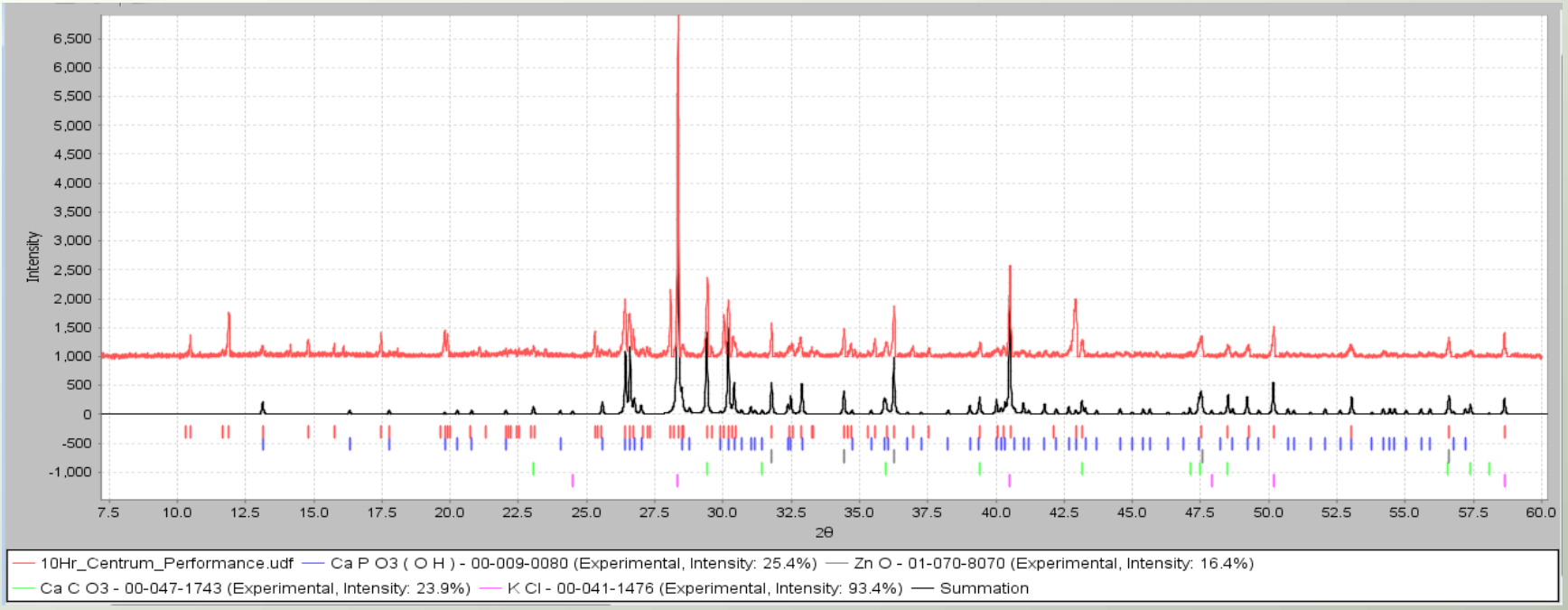
Without using filters there is a high probability that you can get a false positive result.

Centrum Performance®



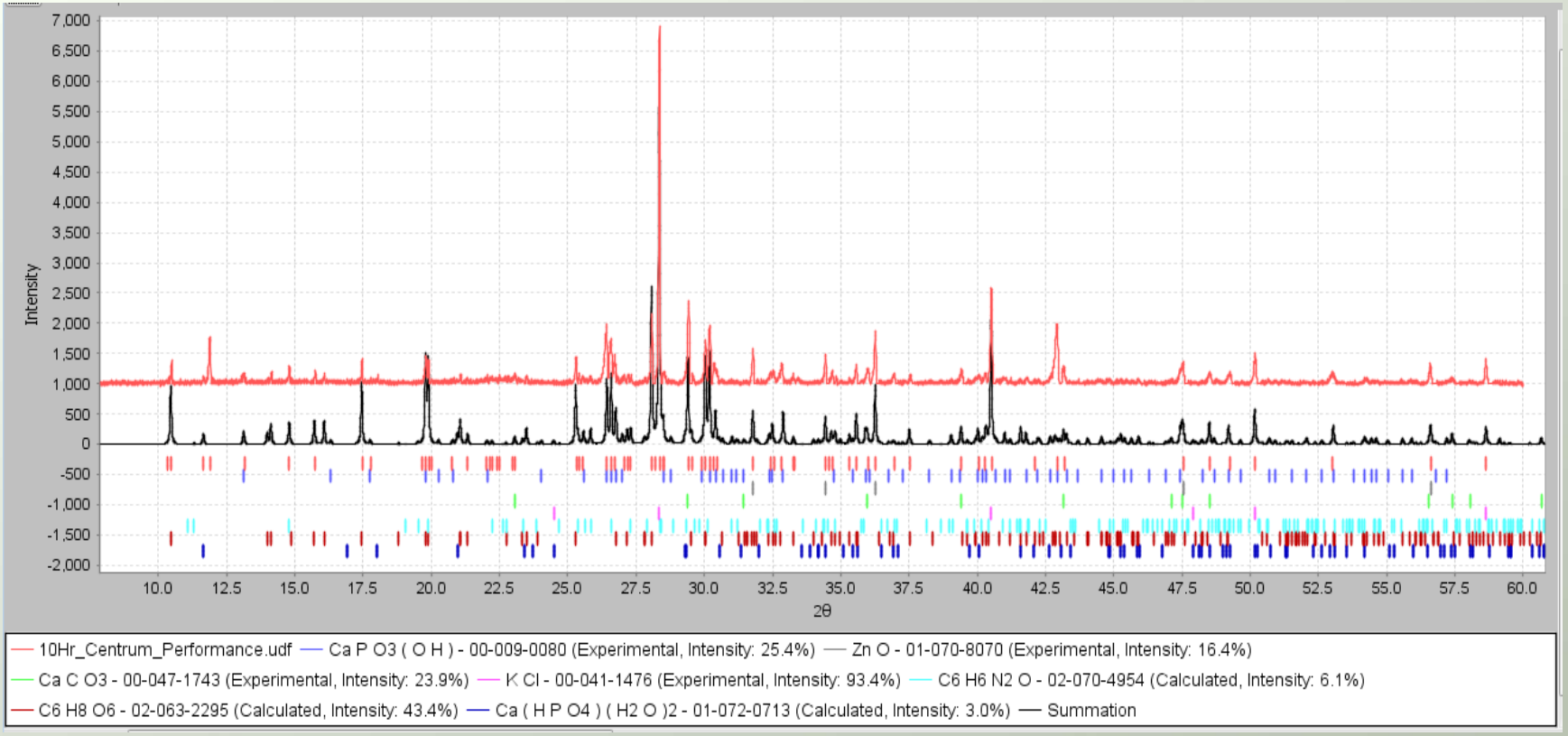
In this example, the very first material identified in a Centrum Performance® Tablet was Calcium Phosphate Hydroxide. This was the first material mentioned in the list of the excipients which are usually ranked in order of concentration.
This material is a commonly used buffering material.
(The red line is the experimental data and the blue the identified reference phase).

Centrum Performance®



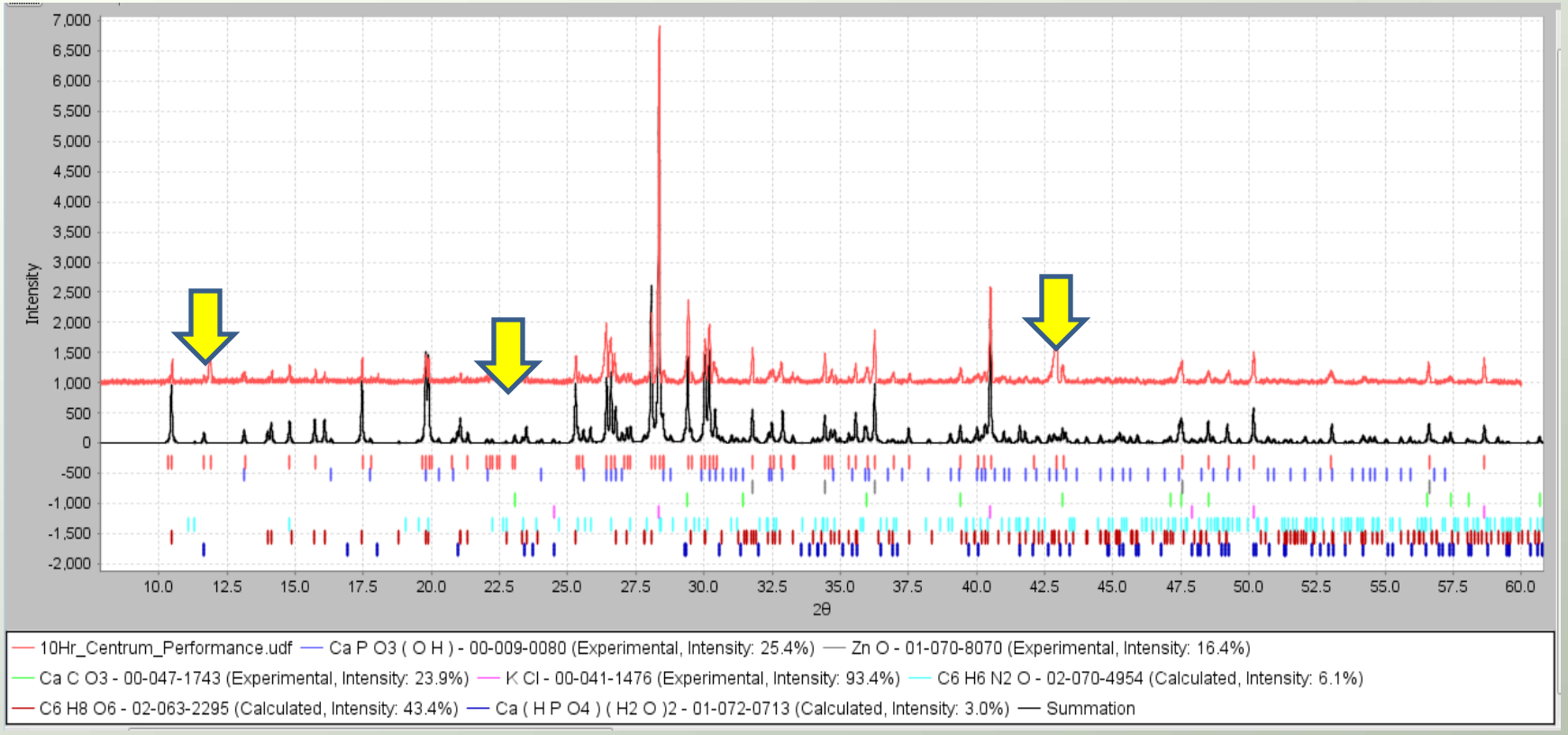
We now use the Pharmaceutical & Excipient subfiles for our database search. This easily identifies the mineral additions to the vitamin pill - calcite, zincite and potassium chloride to go with the original identification of calcium phosphate hydroxide. The above graph compares the scaled four phase match to the experimental data in red. The analysis software (SIeve+) computes that the four phases account for 81% of all the experimental diffraction Intensity.

Centrum Performance®



In this third iteration, once the major phases are identified, the software then identifies niacin, vitamin C and calcium hydroxyphosphate dihydrate. We now have a 7 material identification and have accounted for 88% of the experimental intensity.

Centrum Performance®

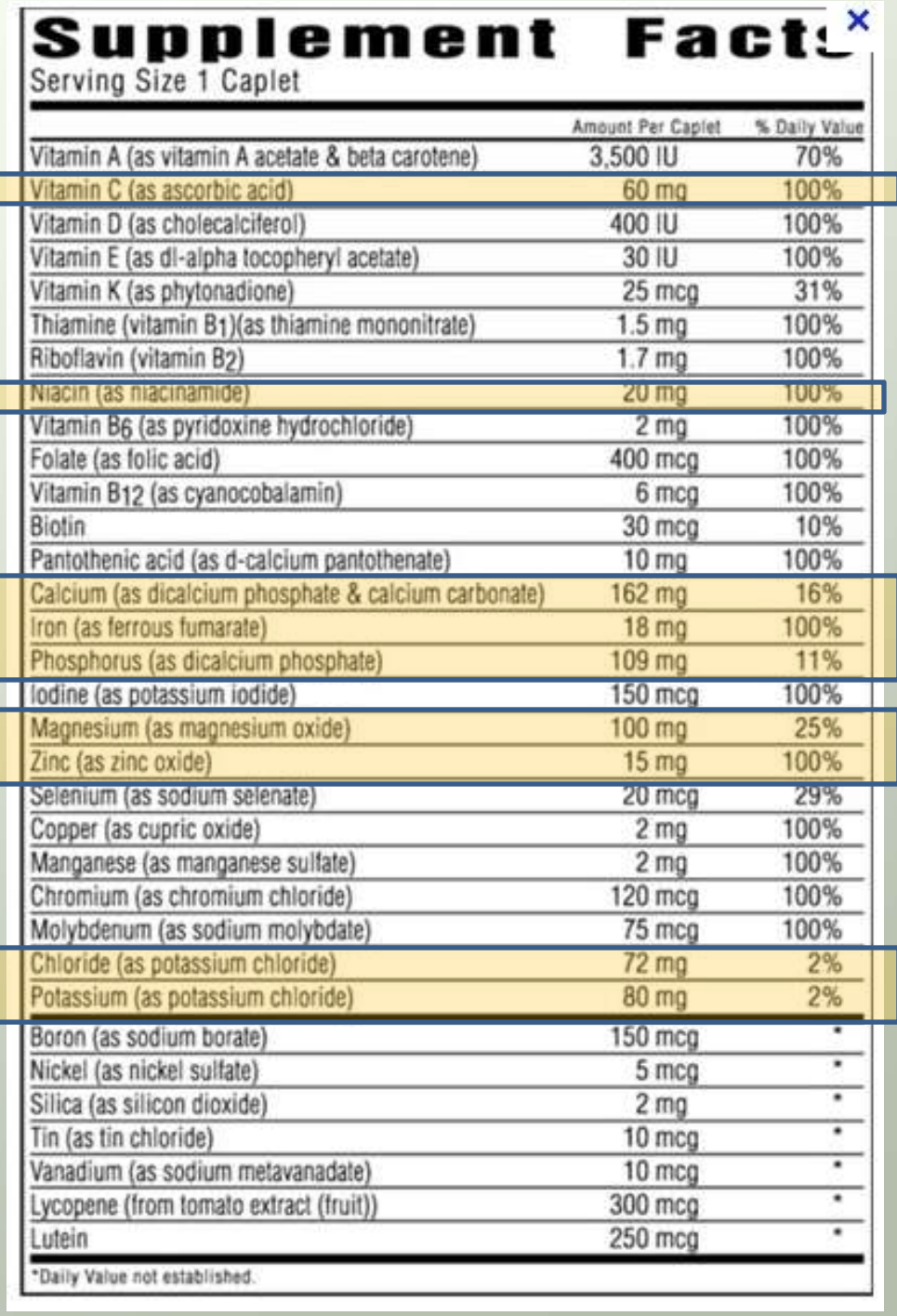


Most automated search/match software would find between 5-7 materials using this data set. However, there are 3 unidentified peaks that are typically not found in normal analyses. Trace identification techniques would identify MgO, iron fumarate and microcrystalline cellulose (see http://www.icdd.com/resources/tutorials/ Data mining-trace phase identification techniques). Overall we identified 10 materials in this formulation.

Formulations

You can see from the prior analyses, we identified all the major ingredients above 20 mg in a 1400 mg pill. This represents 1.4 weight %. In addition, the major excipients of cellulose, calcium hydroxy phosphate and calcium hydroxy phosphate dihydrate were also identified.

We have analyzed a similar tablet at Argonne National Light Source and have been able to detect some of the other B vitamins (Riboflavine, Thiamine, pyridoxine hydrochloride) at concentrations in the tenths of a percent.



Supplement Facts

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Vanadium (as sodium metavanadate)	10 mcg	*
Lycopene (from tomato extract (fruit))	300 mcg	*
Lutein	250 mcg	*

*Daily Value not established.

Centrum Performance®

#		PDF #	Compound Name	I Ratio	I %	I/Ic
1	√	00-009-0080	Calcium Hydroxide Phosphate	0.254	8	
2	√	01-070-8070	Zinc Oxide	0.164	5	5.36
3	√	00-047-1743	Calcium Carbonate	0.239	8	
4	√	00-041-1476	Potassium Chloride	0.934	31	
5	√	02-070-4954	Nicotinamide	0.361	12	0.85
6	√	02-063-2295	L-Ascorbic acid	0.434	14	0.75
7	√	01-072-0713	Calcium Hydrogen Phosphate Hydrate	0.277	9	1.42

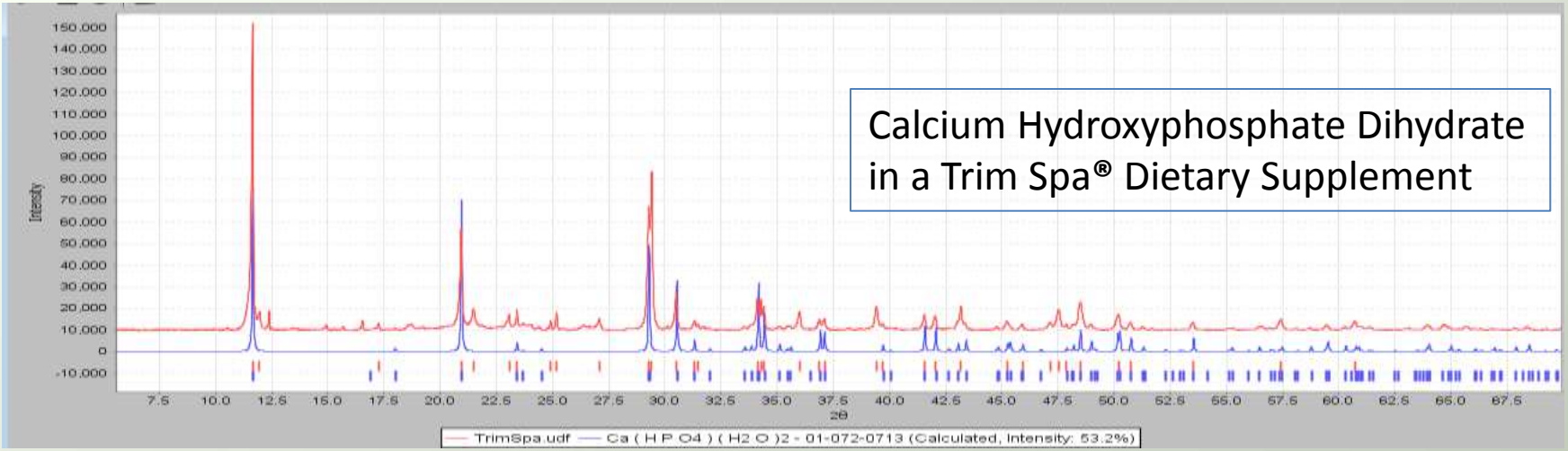
This analysis also demonstrates the power of using a multi-source database like PDF-4/Organics.

1) Three phases were identified using historic powder diffraction data (set 00) from the ICDD. (The three trace phases were also identified from Set 00 – total of 6 phases.)

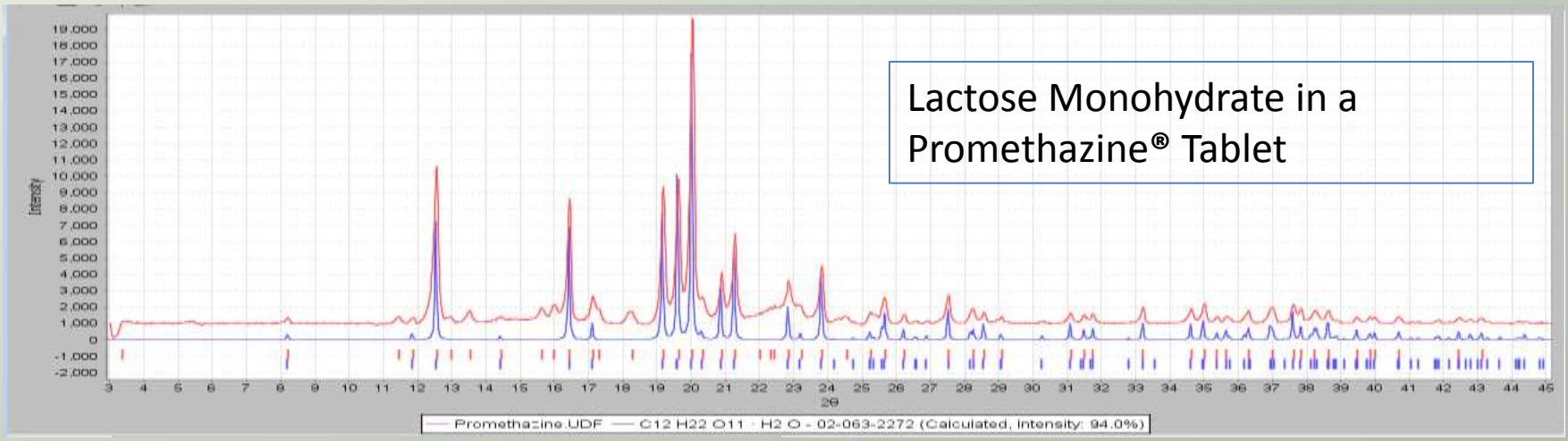
2) Two phases were identified from single crystal data calculated by the ICDD from the CSD. These were the two vitamins.

3) Two phases were identified from single crystal data calculated by the ICDD from the ICSD. These were both excipients.

Common Excipients



Calcium Hydroxyphosphate Dihydrate in a Trim Spa® Dietary Supplement

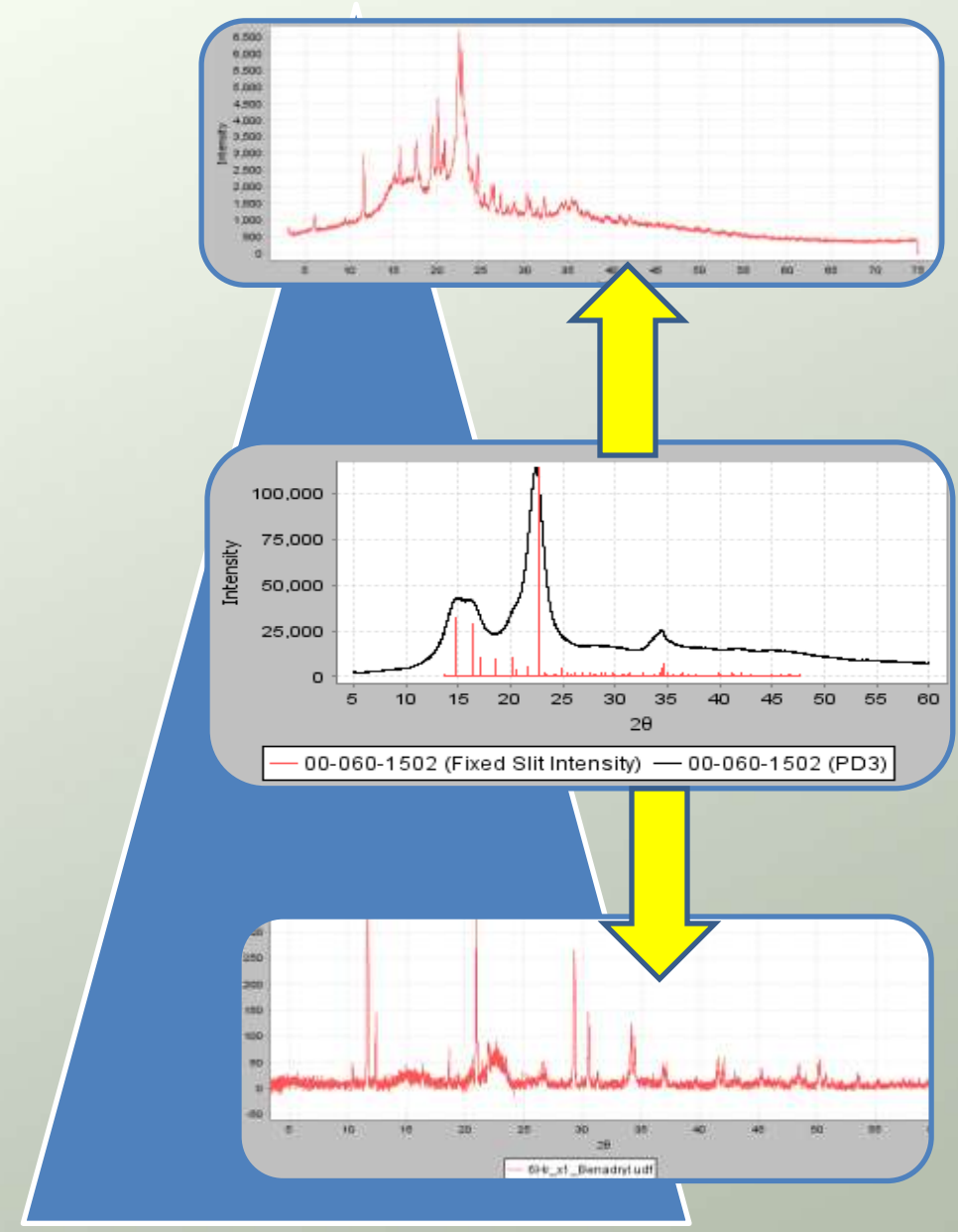


Lactose Monohydrate in a Promethazine® Tablet

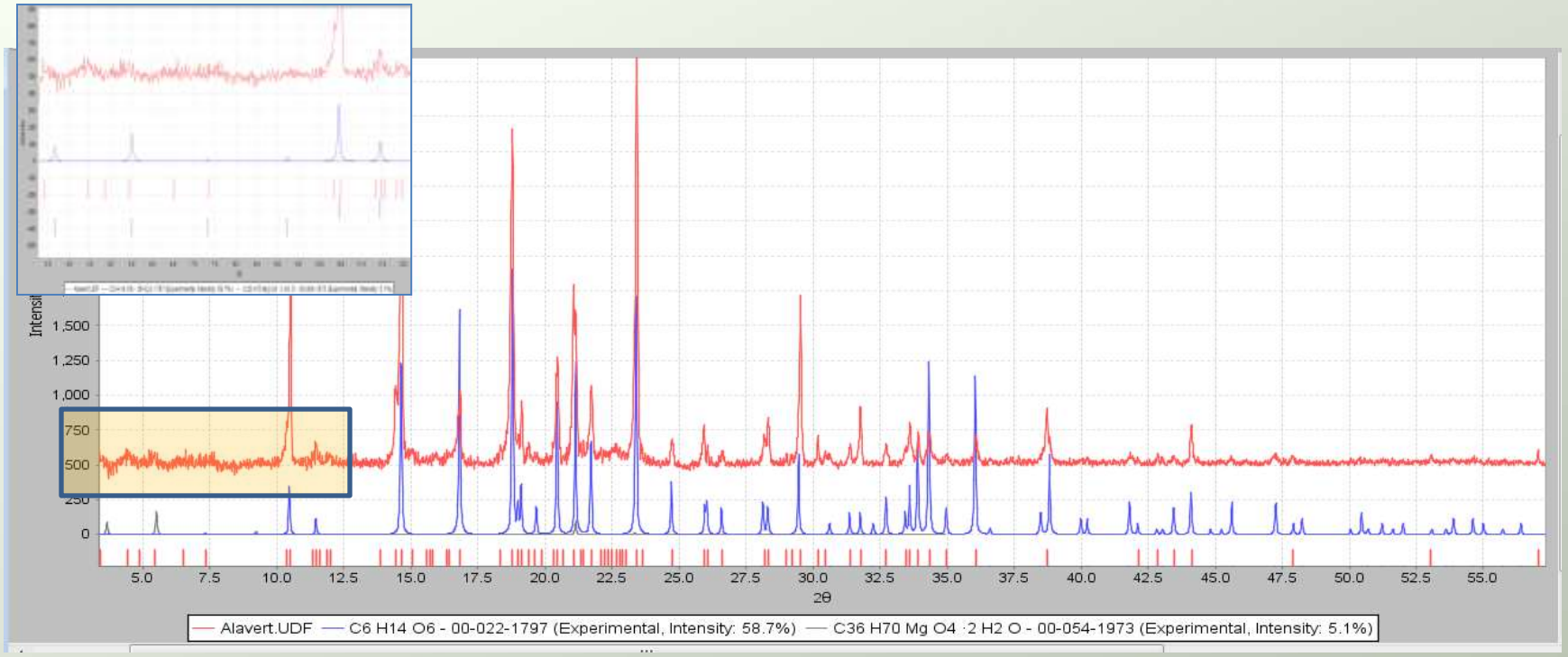
Microcrystalline Cellulose

Microcrystalline cellulose is a *very* common excipient used in many pharmaceutical formulations. It can act as a binder to hold the tablet together and it can also be part of a time release formulation since cellulose can hydrogen bond to many other ingredients and API's.

The middle plot is the ICDD reference for microcrystalline cellulose that was from Sigma-Aldrich. The top pattern is a ground tablet of Pepcid AC® and the bottom pattern is from a ground tablet of Benedryl®. You can see that the identification would be easy in the top pattern and a little more difficult in the bottom pattern. Because of the broad peak profiles microcrystalline cellulose is rarely identified from automated identification programs. It can be identified by total pattern analysis methods and pattern recognition techniques (such as similarity indexes).

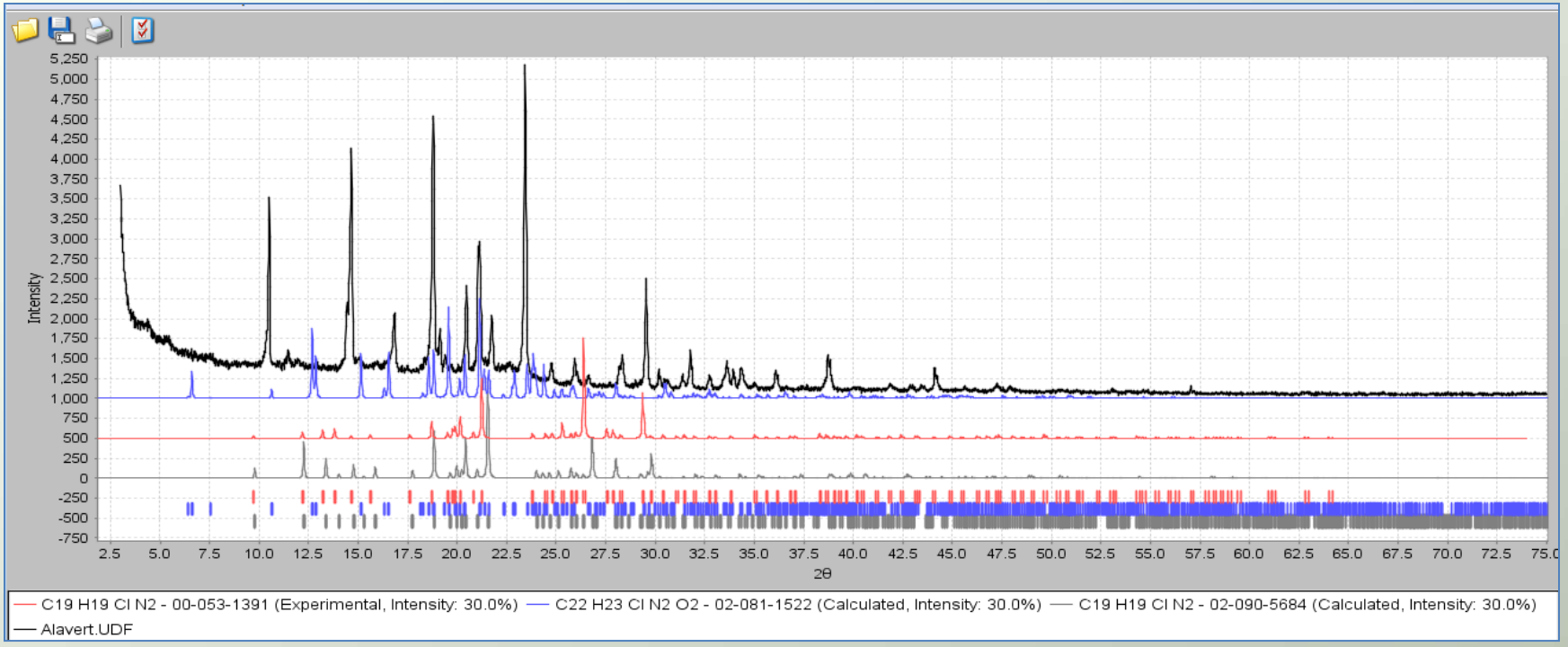


Common Excipients



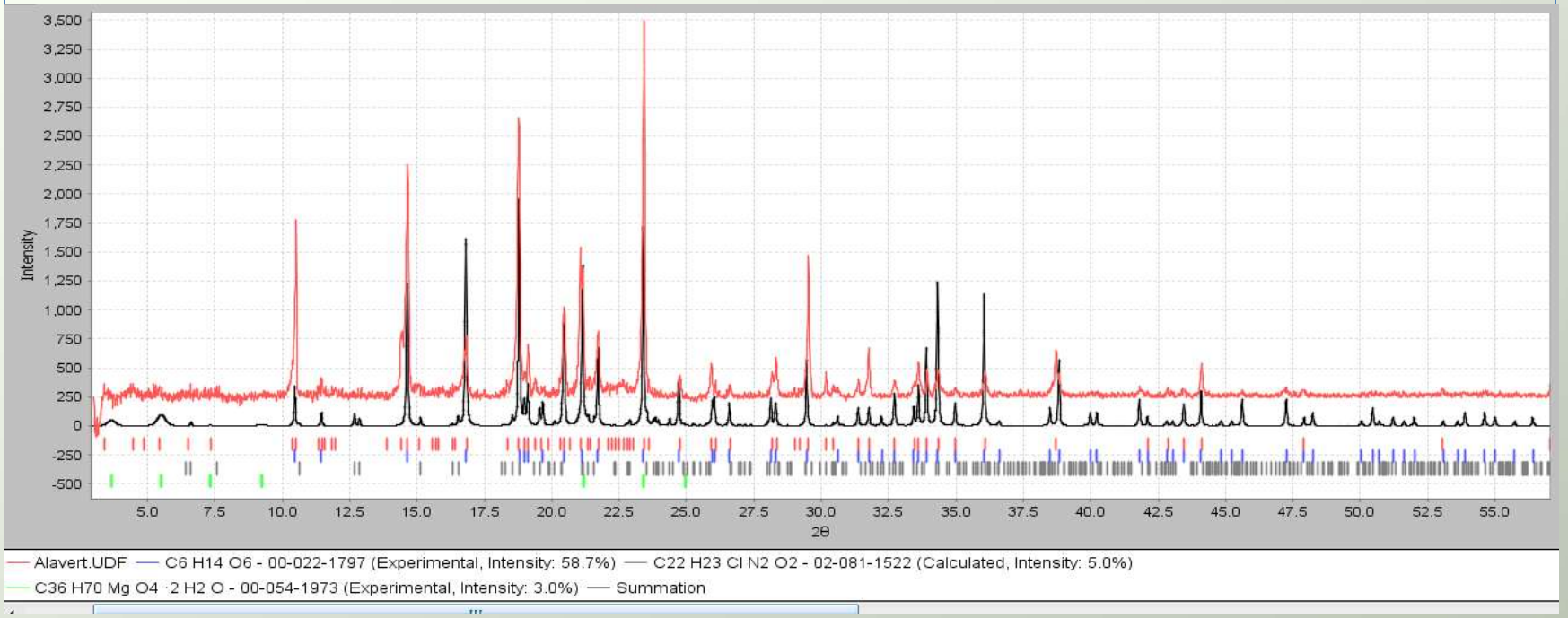
This pattern was produced from a ground tablet of Alavert®. It demonstrates two common excipients. The major phase is D-Mannitol which is used to facilitate the transport of pharmaceuticals to the brain and other parts of the body (Wikipedia) and it is also a sweetener. The second excipient is shown in the highlighted box and is the lubricant magnesium stearate. The stearates have characteristic series of peaks at low angles due to the large unit cell of the linear stearate molecules. This phase is almost always at low concentration, typical of lubricant additions for tablet processing.

API



In the previous slide, we showed a pattern from a ground tablet of Alavert® and the identification of mannitol and magnesium stearate as excipients. The identification of the API, Loratadine, is difficult because the tablet only contains 10 mg of this powerful pharmaceutical. By data mining the database you can compare all the known polymorphs of Loratidine and Desloratidine (3, as shown) and its salts (7) to the experimental data (shown in black). This is useful if you are trying to identify materials of low concentration

Alavert®

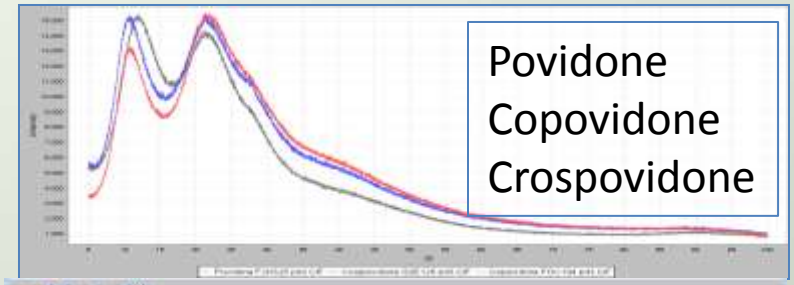


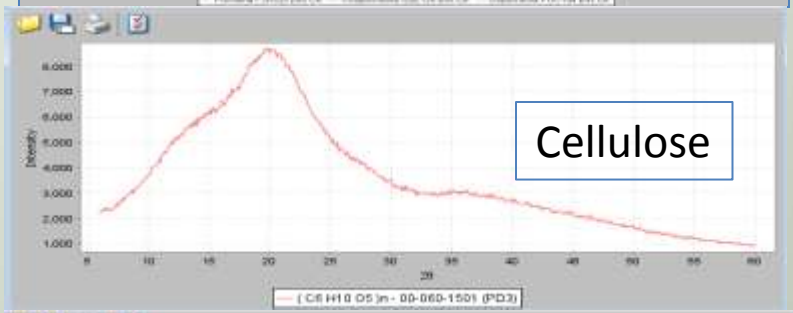
The program SIeve+ identified the specific polymorph of Loratadine contained in Alavert® and now shows the composite solution (black line) compared to the experimental data (red line) for Alavert being composed of D-mannitol, magnesium stearate and Loratine. All three of these ingredients have known polymorphs and the XRD analysis identifies the specific compounds and the specific polymorph. If one looks carefully, there are a few small intensity lines not identified, and trace identification techniques might find more ingredients. The mannitol peak intensities, when compared to the reference, also exhibits some variation, an indication that the mannitol is oriented.

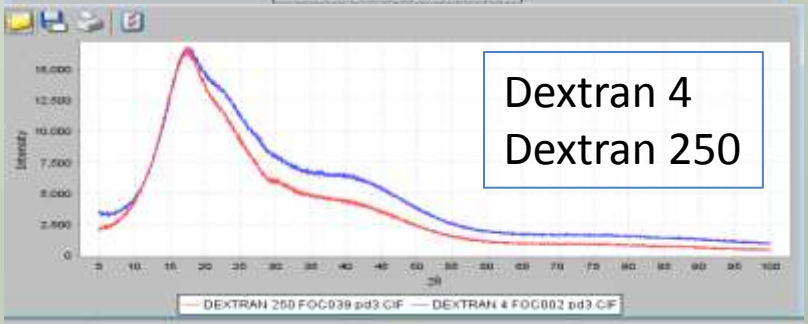
Amorphous Excipients

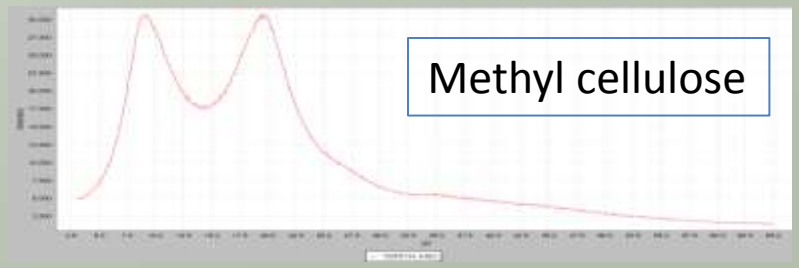
Many formulations contain amorphous excipients. These are difficult to analyze because of their broad diffraction profiles which defeats most automated analysis programs. However, as shown on the right, these materials have distinguishable and reproducible scattering characteristics which means that reference patterns can be used for material identification. They can also be automatically identified in experimental patterns using total pattern matching methods and matching against the known references

Starting in 2010, the ICDD began a program of producing amorphous reference patterns of excipients and commercial materials. The cellulose pattern has been published and the others on this page are scheduled for publication in the PDF-4 databases, Releases 2012 and 2013.









Helpful Hint

This tutorial is designed to present an overview of how to analyze formulated commercial drugs. We did not cover typical errors and sources of errors in the analysis. Like all XRD analyses, the most common types of errors are due to orientation and specimen transparency. As demonstrated by a recent international round robin study, these errors also foil the work of experts. The physics of the root cause of these errors can be found in many XRD textbooks on powder diffraction. Pharmaceutical analyses are plagued by frequent orientation problems caused by crystalline needles and platelets. The low density or organic materials often means that specimen transparency can be appreciable. Fundamentally, these errors can cause your peaks to shift and the intensities to vary, making the analysis difficult if not impossible.

Several very clever scientific software designers have made commercial software that can analyze and correct for these errors. However, the amount of time and expertise required to diagnose and then make the correction, in some cases, can be substantial. With large reference databases (hundreds of millions of d-spacings) you will *always* get an answer. But, if the answer does not make common sense, we recommend that you regrind and remount the specimen and collect another data set. This will often remove or reduce these errors and may practically be much faster than mathematically correcting poor data.

Final Notes

The examples shown in this tutorial were all from commercial drug formulations purchased in grocery stores and pharmacies. We noted places where analyses became difficult and this usually occurred when analyzing non-crystalline ingredients or ingredients that were present in low concentration. In all the examples that were shown, the 2-3 major phases would be easily and automatically identified using a modern diffractometer, good software analysis system and a comprehensive database.

For the latter, we recommend PDF-4/Organics which is the only database in the world specifically designed to analyze these problems. Features of the database include an excipient subfile, pharmaceutical subfile for API's, the addition of thousand of polymeric and inorganic materials so that packaging and production problems can be identified. The database includes the addition of non-crystalline polymers as well as amorphous materials for material identification. The database uses multiple data sources of both powder and single crystal data. The ICDD standardizes all this data for comprehensive coverage and rapid material identification. If you analyze drugs and are also in law enforcement we have a forensic file that has been edited for decades by forensic experts.

The ICDD has a global membership that includes many scientists in the pharmaceutical, specialty chemical, and law enforcement communities that have helped continuously shape both the database and preferred methods of analysis. In this regard, we are grateful to the PPXRD Organizing Committee and the ICDD Members of the Organic and Pharmaceutical Subcommittee.